Remarks/Arguments

Claims 119-121 and 123 are pending and remain rejected in this application. In this submission, Applicants present additional references in support of Applicants' arguments presented in their Appeal Brief of January 26, 2006, and Reply Brief of April 11, 2006, both of which are incorporated by reference herewith in its entirety. Applicants note that some of the references listed in the enclosed Information Disclosure Statement are provided in the form of full-text articles, while others are provided as abstracts. Applicants submit that the full-text articles are provided solely because they are available, and that Applicants do not intend to make any distinction among the references, or to indicate that some references in the IDS are more pertinent or material than others.

Applicants respectfully traverse the present rejections.

Claim Rejections – 35 USC § 101 and 35 USC § 112, first paragraph

Claims 119-121 and 123 remain rejected under 35 U.S.C. §101 allegedly because "the instant application does not disclose a specific and substantial biological role of this protein or its significance."

Claims 119-121 and 123 remain further rejected under 35 U.S.C. §112, first paragraph allegedly "since the claimed invention is not supported by either a specific and substantial asserted utility or a well established utility, one skilled in the art would not know how to use the claimed invention". Applicants respectfully disagree with and traverse these rejections.

Applicants submit, for the reasons set forth in the Appeal Brief filed January 26, 2006 and Reply Brief filed April 11, 2006, both of which are incorporated by reference, that the specification discloses at least one credible, substantial and specific asserted utility for the claimed PRO1281 polypeptides.

As discussed before, Applicants respectfully submitted that Applicants rely on the gene amplification data for patentable utility for the PRO1281 polypeptides, which is clearly disclosed in the instant specification under Example 170. Applicants emphasize that the opinions expressed in the Goddard and Polakis Declaration, are all based on the factual findings of the declarants. For instance, Dr. Polakis explains that in the course of their research using microarray analysis, he and his co-workers identified approximately 200 gene transcripts that are

present in human tumor cells at significantly higher levels than in corresponding normal human cells. Subsequently, antibodies binding to about 30 of these tumor antigens were prepared, and mRNA and protein levels were compared. In approximately 80% of the cases, the researchers found that increases in the level of a particular mRNA correlated with changes in the level of protein expressed from that mRNA when human tumor cells are compared with their corresponding normal cells. Dr. Polakis' statement that "an increased level of mRNA in a tumor cell relative to a normal cell typically correlates to a similar increase in abundance of the encoded protein in the tumor cell relative to the normal cell" is based on such factual, experimental findings, clearly set forth in the Declaration. Without conceding that opinion declarations can be summarily dismissed or not accorded any significant weight, the Declaration of Dr. Polakis would be considered reasonable and accurate by one skilled in the art.

In this submission, Applicants present a second Declaration by Dr. Polakis (Polakis II) that presents evidentiary data in Exhibit B. Exhibit B of the Declaration identifies 28 gene transcripts out of 31 gene transcripts (*i.e.*, greater than 90%) that showed good correlation between tumor mRNA and tumor protein levels. As Dr. Polakis' Declaration (Polakis II) says "[a]s such, in the cases where we have been able to quantitatively measure both (i) mRNA and (ii) protein levels in both (i) tumor tissue and (ii) normal tissue, we have observed that in the vast majority of cases, there is a very strong correlation between increases in mRNA expression and increases in the level of protein encoded by that mRNA." Accordingly, Dr. Polakis has provided the facts to enable the Examiner to draw independent conclusions.

Both Polakis Declarations (Polakis I and II) are further supported by the teachings in Molecular Biology of the Cell, a leading textbook in the field (Bruce Alberts, *et al.*, Molecular Biology of the Cell (3rd ed. 1994) (herein after Cell 3rd) and (4th ed. 2002) (excerpts attached as Exhibit 1). Figure 9-2 of Cell 3rd shows the steps at which eukaryotic gene expression can be controlled. The first step depicted is transcriptional control. Cell 3rd provides that " [f]or most genes transcriptional controls are paramount. This makes sense because, of all the possible control points illustrated in Figure 9-2, only transcriptional control ensures that no superfluous intermediates are synthesized." Cell 3rd at 403 (emphasis added). In addition, the text states that "Although controls on the initiation of gene transcription are the predominant form of regulation for most genes, other controls can act later in the pathway from RNA to protein to modulate the

amount of gene product that is made." Cell 3rd at 453 (emphasis added). Thus, as established in Cell 3rd, the predominant mechanism for regulating the amount of protein produced is by regulating transcription initiation.

In Cell 4th, Figure 6-3 on page 302 illustrates the basic principle that there is a correlation between increased gene expression and increased protein expression. The accompanying text states that "a cell can change (or regulate) the expression of each of its genes according to the needs of the moment – *most obviously by controlling the production of its mRNA.*" Cell 4th at 302 (Emphasis added). Similarly, Figure 6-90 on page 364 of Cell 4th illustrates the path from gene to protein. The accompanying text states that while potentially each step can be regulated by the cell, "the initiation of transcription is the most common point for a cell to regulate the expression of each of its genes." Cell 4th at 364 (emphasis added). This point is repeated on page 379, where the authors state that of all the possible points for regulating protein expression, "[f]or most genes transcriptional controls are paramount." Cell 4th at 379 (Emphasis added).

Further support for Applicants' position can be found in the textbook, Genes VI, (Benjamin Lewin, Genes VI (1997)) (copy enclosed under Exhibit 1) which states "having acknowledged that control of gene expression can occur at multiple stages, and that production of RNA cannot inevitably be equated with production of protein, it is clear that the overwhelming majority of regulatory events occur at the initiation of transcription." *Genes VI* at 847-848 (Emphasis added).

Additional support is also found in Zhigang *et al.*, World Journal of Surgical Oncology 2:13, 2004 (copy enclosed in Exhibit 1). Zhigang studied the expression of prostate stem cell antigen (PSCA) protein and mRNA to validate it as a potential molecular target for diagnosis and treatment of human prostate cancer. The data showed "a high degree of correlation between PSCA protein and mRNA expression" *Zhigang* at 4. Of the samples tested, 81 out of 87 showed a high degree of correlation between mRNA expression and protein expression. The authors conclude that "it is demonstrated that PSCA protein and mRNA overexpressed in human prostate cancer, and that the increased protein level of PSCA was resulted from the upregulated transcription of its mRNA." *Zhigang* at 6. Even though the correlation between mRNA expression and protein expression occurred in 93% of the samples tested, not 100%, the authors

state that "PSCA may be a promising molecular marker for the clinical prognosis of human Pca and a valuable target for diagnosis and therapy of this tumor." *Id.* at 7

Further, Meric *et al.*, Molecular Cancer Therapeutics, vol. 1, 971-979 (2002) (a copy enclosed in Exhibit 1) states the following:

> The **fundamental principle** of molecular therapeutics in cancer is to exploit the differences in gene expression between cancer cells and normal cells...[M]ost efforts have concentrated on identifying differences in gene expression at the level of mRNA, which can be attributable to either DNA amplification or to differences in transcription. Meric *et al.* at 971 (Emphasis added).

Those of skill in the art would not be focusing on differences in gene expression between cancer cells and normal cells if there were no correlation between gene expression and protein expression.

Together, the Declarations of Polakis, the accompanying references, and the excerpts and references provided above all establish that the accepted understanding in the art is that there is a reasonable correlation between changes in gene expression and the level of the encoded protein.

In addition to the supporting references previously submitted by Applicants, Applicants submit the following references to further support the assertion that changes in mRNA levels generally lead to corresponding changes in the level of the encoded polypeptide.

In a comprehensive study by Orntoft *et al.* (Mol. Cell. Proteomics. 2002; 1(1):37-45) (copy enclosed as Exhibit 2), the authors examined gene amplification, mRNA expression level, and protein expression in pairs of non-invasive and invasive human bladder tumors. *Id.* at Abstract. The authors examined 40 well resolved abundant known proteins, and found that "[i]n general there was a highly significant correlation (p<0.005) between mRNA and protein alterations. Only one gene showed disagreement between transcript alteration and protein alteration." *Id.* at 42, col. 2. The alternations in mRNA and protein included both increases and decreases. *Id.* at 43, Table II. Clearly, a correlation in 39 of 40 genes examined supports Applicants' assertion that changes in mRNA level generally lead to corresponding changes in protein level.

In a study by Wang *et al.* (Urol. Res. 2000; 28(5):308-15) (abstract attached as Exhibit 3) the authors report that down-regulation of E-cadherin protein has been shown in various human

tumors. *Id.* at Abstract. In the reported study, the authors examined the expression of cadherins and associated catenins at the mRNA level in paired tumor and nonneoplastic primary prostate cultures. They report that "[s]ix of seven cases of neoplastic cultures showed moderately-to-markedly decreased levels of E-cadherin and P-cadherin mRNA. Similar losses of alpha-catenin and beta-catenin mRNA were also observed." *Id.* As Applicants' assertion would predict, the authors state that the mRNA measures showed "good correlation" with the results from protein measures. The authors conclude by stating that "this paper presents a coordinated down-regulation in the expression of E-cadherin and associated catenins at the mRNA and protein level in most of the cases studied." *Id.*

In a more recent study by Munaut *et al.* (Int. J. Cancer. 2003; 106(6):848-55) (abstract attached as Exhibit 4) the authors report that vascular endothelial growth factor (VEGF) is expressed in 64-95% of glioblastomas (GBMs), and that VEGF receptors (VEGFR-1, its soluble form sVEGFR-1, VEGFR-2 and neuropilin-1) are expressed predominantly by endothelial cells. *Id.* at Abstract. The authors explain that infiltrating tumor cells and newly-formed capillaries progress through the extracellular matrix by local proteolysis involving matrix metalloproteinases (MMPs). In the present study, the authors "used quantitative RT-PCR, Western blot, gelatin zymography and immunohistochemistry to study the expression of VEGF, VEGFR-1, VEGFR-2, sVEGFR-1, neuropilin-1, MT1-MMP, MMP-2, MMP-9 and TIMP-2 in 20 human GBMs and 5 normal brains. The expression of these MMPs was markedly increased in most GBMs with excellent correlation between mRNA and protein levels." *Id.* Thus, the results support Applicants' assertion that changes in mRNA level lead to corresponding changes in protein level.

In another recent study, Hui *et al.* (Leuk. Lymphoma. 2003; 44(8):1385-94 (abstract attached as Exhibit 5) used real-time quantitative PCR and immunohistochemistry to evaluate cyclin D1 mRNA and protein expression levels in mantle cell lymphoma (MCL). *Id.* at Abstract. The authors report that seven of nine cases of possible MCL showed overexpression of cyclin D1 mRNA, while two cases showed no cyclin D1 mRNA increase. *Id.* Similarly, "[s]ix of the seven cyclin D1 mRNA overexpressing cases showed increased cyclin D1 protein on tissue array immunohistochemistry; one was technically suboptimal." *Id.* The authors conclude that the

study "demonstrates good correlation and comparability between measure of cyclin D1 mRNA ... and cyclin D1 protein." *Id.* Thus, this reference supports Applicants' assertion.

In a recent study by Khal *et al.* (Int. J. Biochem. Cell Biol. 2005; 37(10):2196-206) (abstract attached as Exhibit 6) the authors report that atrophy of skeletal muscle is common in patients with cancer and results in increased morbidity and mortality. *Id.* at Abstract. To further understand the underlying mechanism, the authors studied the expression of the ubiquitin-proteasome pathway in cancer patient muscle using a competitive RT-PCR to measure expression of mRNA for proteasome subunits C2 and C5, while protein expression was determined by western blotting. "Overall, both C2 and C5 gene expression was increased by about three-fold in skeletal muscle of cachectic cancer patients (average weight loss 14.5+/-2.5%), compared with that in patients without weight loss, with or without cancer. ... There was a good correlation between expression of proteasome 20Salpha subunits, detected by western blotting, and C2 and C5 mRNA, showing that increased gene expression resulted in increased protein synthesis." These findings support Applicants' assertion that changes in mRNA level lead to changes in protein level.

Maruyama *et al.* (Am. J. Patho. 1999; 155(3):815-22) (abstract attached as Exhibit 7) investigated the expression of three Id proteins (Id-1, Id-2 and Id-3) in normal pancreas, in pancreatic cancer and in chronic pancreatitis (CP). The authors report that pancreatic cancer cell lines frequently coexpressed all three Ids, "exhibiting good correlation between Id mRNA and protein levels." *Id.* at Abstract. In addition, the authors teach that all three Id mRNA levels were expressed at high levels in pancreatic cancer samples compared to normal or CP samples. At the protein level, Id-1 and Id-2 staining was faint in normal tissue, while Id-3 ranged from weak to strong. In contrast, in the cancer tissues "many of the cancer cells exhibited abundant Id-1, Id-2, and Id-3 immunoreactivity," and Id-1 and Id-2 protein was increased significantly in the cancer cells by comparison to the respective controls, mirroring the overexpression at the mRNA level. Thus, the authors report that in both cell lines and tissue samples, increased mRNA levels leads to an increase in protein overexpression, supporting Applicants' assertion.

Support for Applicants' assertion is also found in an article by Caberlotto *et al.* (Neurosci. Lett. 1999; 256(3):191-4) (abstract attached as Exhibit 8). In a previous study, the authors investigated alterations of neuropeptide Y (NPY) mRNA expression in the Flinders Sensitive

Line rats (FSL), an animal model of depression. *Id.* at Abstract. The authors reported that in the current study, that NPY-like immunoreactivity (NPY-LI) was decreased in the hippocampal CA region, and increased in the arcuate nucleus, and that fluoxetine treatment elevated NPY-LI in the arcuate and anterior cingulate cortex. The authors state that "[t]he results demonstrate a good correlation between NPY peptide and mRNA expression." Thus, increases and decreases in mRNA levels were reflected in corresponding changes in protein level.

Misrachi and Shemesh (Biol. Reprod. 1999; 61(3):776-84) (abstract attached as Exhibit 9) investigated their hypothesis that FSH regulates the bovine cervical prostaglandin E(2) (PGE(2)) synthesis that is known to be associated with cervical relaxation and opening at the time of estrus. *Id.* at Abstract. Cervical tissue from pre-estrous/estrous, luteal, and postovulatory cows were examined for the presence of bovine (b) FSH receptor (R) and its corresponding mRNA. The authors report that bFSHR mRNA in the cervix was maximal during pre-estrus/estrus, and that the level of FSHR protein was significantly higher in pre-estrous/estrous cervix than in other cervical tissues. *Id.* The authors state that "[t]here was a good correlation between the 75-kDa protein expression and its corresponding transcript of 2.55 kb throughout the estrous cycle as described by Northern blot analysis as well as RT-PCR." *Id.* Thus, changes in the level of mRNA for bFSHR led to corresponding changes in FSHR protein levels, a result which supports Applicants' assertion.

In a study by Stein *et al.* (J. Urol. 2000; 164(3 Pt 2):1026-30) (abstract attached as Exhibit 10), the authors studied the role of the regulation of calcium ion homeostasis in smooth muscle contractility. *Id.* at Abstract. The authors investigated the correlation between sarcoplasmic endoplasmic reticulum, calcium, magnesium, adenosine triphosphatase (SERCA) protein and gene expression, and the contractile properties in the same bladder. Partial bladder outlet obstructions were created in adult New Zealand white rabbits, which were divided into control, sham operated and obstructed groups. Stein *et al.* report that "[t]he relative intensities of signals for the Western [protein] and Northern [mRNA] blots demonstrated a strong correlation between protein and gene expression. The loss of SERCA protein expression is mediated by down-regulation in gene expression in the same bladder." *Id.* This report supports Applicants' assertion that changes in mRNA level, *e.g.,* a decrease, lead to a corresponding change in the level of the encoded protein, *e.g.,* a decrease.

9

In an article by Gou and Xie (Zhonghua Jie He He Hu Xi Za Zhi. 2002; 25(6):337-40) (abstract attached as Exhibit 11) the authors investigated the expression of macrophage migration inhibitory factor (MIF) in human acute respiratory distress syndrome(ARDS) by examining the expression of MIF mRNA and protein in lung tissue in ARDS and normal persons. *Id.* at Abstract. The authors report "undetectable or weak MIF mRNA and protein expression in normal lungs. In contrast, there was marked upregulation of MIF mRNA and protein expression in the ARDS lungs." *Id.* This is consistent with Applicants' assertion that a change in mRNA for a particular gene, *e.g.,* an increase, generally leads to a corresponding change in the level of protein expression, *e.g.,* an increase.

These studies are representative of <u>numerous</u> published studies which support Applicants' assertion that changes in mRNA level generally lead to corresponding changes in the level of the expressed protein. Applicants submit herewith an addition 70 references (abstracts attached as Exhibit 12) which support Applicants' assertion.

In addition to these supporting references, Applicants also submit herewith additional references which offer support of Applicants' asserted utility.

For example, in an article by Futcher *et al.* (Mol. Cell Biol. 1999; 19(11):7357-68) (abstract attached as Exhibit 13) the authors conducted a study of mRNA and protein expression in yeast. Futcher *et al.* report "a good correlation between protein abundance, mRNA abundance, and codon bias." *Id.* at Abstract.

In a study which is more closely related to Applicants' asserted utility, Godbout *et al.* (J. Biol. Chem. 1998; 273(33)21161-8) (abstract attached as Exhibit 14) studied the DEAD box gene, DDX1, in retinoblastoma and neuroblastoma tumor cell lines. The authors report that "there is a good correlation with DDX1 gene copy number, DDX1 transcript levels, and DDX1 protein levels in all cell lines studied." *Id.* Thus, in these cancer cell lines, DDX1 mRNA and protein levels are correlated.

Similarly, in an article by Papotti *et al.* (Virchows Arch. 2002; 440(5):461-75) (abstract attached as Exhibit 15) the authors examined the expression of three somatostatin receptors (SSTR) at the mRNA and protein level in forty-six tumors. *Id.* at Abstract. The authors report a "good correlation between RT-PCR [mRNA level] and IHC [protein level] data on SSTR types 2, 3, and 5." *Id.*

Van der Wilt *et al.* (Eur. J. Cancer. 2003; 39(5):691-7) (abstract attached as Exhibit 16) studied deoxycytidine kinase (dCK) in seven cell lines, sixteen acute myeloid leukemia samples, ten human liver samples, and eleven human liver metastases of colorectal cancer origin. *Id.* at Abstract. The authors report that "enzyme activity and protein expression levels of dCK in cell lines were closely related to the mRNA expression levels" and that there was a "good correlation between the different dCK measurements in malignant cells and tumors." *Id.*

Grenback *et al.* (Regul. Pept. 2004; 117(2):127-39) (abstract attached as Exhibit 17) studied the level of galanin in human pituitary adenomas using a specific radioimmunoassay. *Id.* at Abstract. The authors report that "[i]n the tumors analyzed with in situ hybridization there was a good correlation between galanin peptide levels and galanin mRNA expression." *Id.*

Similarly, Shen *et al.* (Blood. 2004; 104(9):2936-9) (abstract attached as Exhibit 18) examined the level of B-cell lymphoma 2 (BCL2) protein expression in germinal center (GC) B-cells and diffuse large B-cell lymphoma (DLBCL). *Id.* at Abstract. The authors report that "GC cells had low expression commensurate with the low protein expression level" and that in DLBCL the level of BCL2 mRNA and protein expression showed "in general, a good correlation." *Id.*

Likewise, in an article by Fu *et al.* (Blood 2005; 106(13):4315-21) (abstract attached as Exhibit 19) the authors report that six mantle cell lymphomas studied "expressed either cyclin D2 (2 cases) or cyclin D3 (4 cases). " *Id.* at Abstract. "There was a good correlation between cyclin D protein expression and the corresponding mRNA expression levels by gene expression analysis." *Id.*

These examples are only a few of the many references Applicants could cite in rebuttal to the PTO's arguments. Applicants submit herewith 26 additional references (abstracts attached as Exhibit 20) which also support Applicants' assertion in that they report a correlation between the level of mRNA and corresponding protein, contrary to the assertion of the PTO that mRNA and protein levels are not correlated.

In summary, Applicants submit herewith a total of 148 references in addition to the declarations and references already of record which support Applicants' asserted utility. These references support the assertion that in general, a change in mRNA expression level for a particular gene leads to a corresponding change in the level of expression of the encoded protein.

11

As Applicants have previously acknowledged, the correlation between changes in mRNA level and protein level is not exact, and there are exceptions (*see, e.g.,* abstracts attached as Exhibit 21). However, Applicants remind the PTO that the asserted utility does not have to be established to a statistical certainty, or beyond a reasonable doubt. *See M.P.E.P.* at § 2107.02, part VII (2004). Therefore, the fact that there are exceptions to the correlation between changes in mRNA and changes in protein does not provide a proper basis for rejecting Applicants' asserted utility. Applicants submit that considering the evidence as a whole, with the overwhelming majority of the evidence supporting Applicants' asserted utility, a person of skill in the art would conclude that Applicants' asserted utility is "more likely than not true." *Id.*
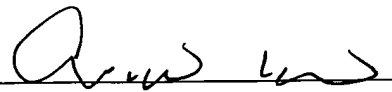
Applicants further submit that based on the claimed utility for PRO1281 polypeptides and its antibodies in the diagnosis of colon cancer, and the collective teachings in the specification, one of skill in the art would know exactly how to make and use the claimed antibodies for the diagnosis of colon cancer. Accordingly, the present 35 U.S.C. §101 and §112, first paragraph utility and enablement rejections should be withdrawn.

The present application is believed to be in *prima facie* condition for allowance, and an early action to that effect is respectfully solicited.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 08-1641 (Attorney Docket No.: 39780-2730P1C26). Please direct any calls in connection with this application to the undersigned at the number provided below.

Respectfully submitted,

Date: August 2, 2006

Ginger R. Dreger
Reg. No. 33,055

HELLER EHRMAN, LLP
Customer No. 35489
275 Middlefield Road
Menlo Park, California 94025
Telephone: (650) 324-7000
Facsimile: (650) 324-0638